Iron--Molybdenum--Sulphur Clusters

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Iron–molybdenum–sulphur clusters

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The recognition that molybdenum is present in the nitrogenase enzymes as an extractable cofactor, containing iron, molybdenum and sulphur in the ratios 6–8 : 1 : 4–6, has stimulated investigations concerned with the synthesis and characterization of compounds containing these three elements. This paper describes the preparation and structure of complexes containing an Fe–Mo–S framework, most of these systems being classified according to whether they contain a MoS_4 group coordinated as a bidentate ligand to one or two iron atoms, or an Fe_3MoS_4 cubane-like cluster. Several physical properties of these complexes are presented, with reference to the corresponding properties of the iron–molybdenum cofactor of the nitrogenases, especially for those complexes that contain a pair of Fe_3MoS_4 cubane-like clusters.

Introduction

The nitrogenase enzymes occur in both symbiotic and free-living organisms that grow either aerobically or anaerobically. The most studied nitrogenases are those from free-living heterotrophs since these are most readily available and can be cultured on a reasonably large scale in the laboratory. In all cases so far investigated, nitrogenase is constituted of two proteins: a ferredoxin protein, of relative molecular mass *ca.* 60000 with an $\{Fe_4S_4(Scys)_4\}$ centre, and a molybdoferredoxin protein, of relative molecular mass *ca.* 220000 with approximately 30–32 Fe, 30–32 S^{2-}, and 2Mo per molecule. The ferredoxin mediates electron transfer to the molybdoferredoxin, this transfer requiring the hydrolysis of ATP, and the latter protein contains the catalytic site(s) for the coupled proton–electron transfers to the enzyme's substrates (Hardy *et al.* 1977; Mortenson & Thorneley 1979; Gibson & Newton 1981). The results of extrusion (Shah & Brill 1977; Kurtz *et al.* 1979) and spectroscopic (Rawlings *et al.* 1978; Huynh *et al.* 1980) studies are consistent with the metal atoms of the molybdoferredoxin porteins occurring as four Fe_4S_4 (or P-type) centres and two iron-molybdenum cofactor (FeMoco), $Fe_{6-8}MoS_{4-6}$ (or M-type) centres; another centre (labelled S), accounting for some 5% of the iron, may also be present. The generally held view is that the FeMoco centre(s) represent the site(s) for substrate reduction, and this view is reinforced by the inactivity of the *Azotobacter vinelandii* UW45 mutant protein that lacks these centres (Shah & Brill 1977).

An important aspect of the characterization of the M-type centre and the *N*-methylformamide-extracted cofactor (FeMoco) has been their e.p.r. spectra, which correspond to an $S = \frac{3}{2}$ system, with principal apparent *g* values of *ca.* 4.6, 3.3 and 2.0. ^{57}Fe Mössbauer spectroscopic studies have shown that the native protein and FeMoco contain one $S = \frac{3}{2}$ centre per molybednum, each

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magnetic centre containing about six iron atoms in a spin-coupled structure (Rawlings *et al.* 1978). This magnetic centre may correspond to the moiety termed the 'molybdenum–iron cluster', which contains about six iron atoms per molybdenum, isolated from the molybdoferredoxin of *Azotobacter vinelandii* by methyl ethyl ketone extraction, the characteristic $M_{\mathrm{e.p.r.}}$ ($S = \frac{3}{2}$) signal being manifest when the cluster is transferred from methyl ethyl ketone to *N*-methylformamide. This 'molybdenum–iron cluster' seems to have (two) less iron atoms than FeMoco and, unlike the latter, fails to activate the UW45 mutant protein (Shah & Brill 1981). The $S = \frac{3}{2}$ level is an intermediate redox state of the FeMoco and M-type centres; oxidation and reduction lead to e.p.r.-silent states and, in the protein, the reduced state is believed to correspond to the catalytically active condition (Burgess *et al.* 1980).

The measurement and interpretation of the molybdenum K-edge X-ray absorption spectrum of the molybdoferredoxin of *Azotobacter vinelandii* and the *N*-methylformamide-extracted cofactor (Burgess *et al.* 1981; S. D. Conradson & K. O. Hodgson, personal communication 1982) have provided a more definitive characterization of the molybdenum environment than the initial investigation (Cramer *et al.* 1978), which was so important in stimulating chemical endeavours to prepare iron–molybdenum–sulphur clusters as potential analogues of FeMoco. The latest results are consistent with relatively little perturbation of the cofactor upon extrusion and the extended X-ray absorption fine structure (e.X.a.f.s.) for reduced FeMoco can be interpreted by a molybdenum environment consisting of 3 oxygen (or nitrogen) atoms at 2.10 Å†, 3 sulphur atoms at 2.36 Å, and 2.8 iron atoms at 2.68 Å.

PREPARATION AND STRUCTURE OF Fe–Mo–S COMPLEXES

Complexes that possess an Fe–Mo–S framework are conveniently classified according to whether they involve (1) a tetrathiomolybdate group coordinated to one or two iron atoms, or (2) an Fe_3MoS_4 cubane-like cluster. The majority of the syntheses to the complexes of type 1 (Coucouvanis 1981) have involved a reaction between an iron(II) complex and a $[MoS_4]^{2-}$ salt, whereas the formation of Fe_3MoS_4 cubane-like cluster complexes has been achieved by reacting $FeCl_3$ with a $[MoS_4]^{2-}$ salt, in the presence of sufficient thiolate for ligation and reduction of the metal atoms (Christou & Garner 1980*a*; Holm 1981 and references therein). The only Fe–Mo–S complex currently known that does not fit into this classification is $[\{(C_2H_4S_2)\,Mo(S)\,S_2\}_2Fe]^{3-}$ (Dahlstrom *et al.* 1981).

Each of the synthetic routes currently available for the formation of type 2 complexes yields a dimer, consisting of two $\{(RSFe)_3MoS_4\}$ cubane-like clusters linked across their molybdenum centres by a $(\mu_2\text{-OMe})_3$, as in $[Fe_6Mo_2S_8(SPh)_6(OMe)_3]^{3-}$, a $(\mu_2\text{–SR})_3$, as in $[Fe_6Mo_2S_8(SR)_9]^{3-}$ (R = e.g. Et, CH_2CH_2OH, Ph, C_6H_4–4–X; X = H, Me, or Cl) (see figure 1), a $(\mu_2\text{–S})\,(\mu_2\text{–SR})_2$, as in $[Fe_6Mo_2S_9(SEt)_8]^{3-}$, or a $(\mu_2\text{–SR})_3Fe^{\mathrm{II\ or\ III}}(\mu_2\text{–SR})_3$ as in $[Fe_7Mo_2S_8(SR)_{12}]^{4-\ \mathrm{or}\ 3-}$ (R = e.g. Et or CH_2Ph) arrangement. Some ligand substitution reactions of these clusters have been developed, and the terminal, iron-bound thiolates shown to be readily replaced by other thiols (R′S) or halides (X = Cl or Br), by treatment with R′SH or PhCOX, respectively (Christou & Garner 1980*b*; Palermo *et al.* 1982). The molybdenum centres are kinetically inert to such reactions and it has so far only been possible to achieve substitution thereon via the $(\mu_2\text{–SR})_3Fe(\mu_2\text{–SR})_3$ complexes; reactions with catechols have been developed to produce not only complexes with a single Fe_3MoS_4 cluster but also an important and versatile substitution chemistry at the molybdenum.

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† 1Å = 10^{-10} m = 10^{-1} nm.

The formation of complexes containing Fe–W–S clusters has proceeded in parallel with the corresponding studies for the Fe–Mo–S clusters. Comparisons between such complexes of these two metals are important because tungsten, although incorporated into the molybdoferredoxin of nitrogenase (Benemann *et al.* 1973), does not lead to a functional enzyme (Shah & Brill 1977, 1981). The structural data obtained for type 1 and type 2 Fe–M–S (M = Mo or W) complexes indicate that the substitution of tungsten for molybdenum produces only a small change in dimensions for type 1 and an insignificant change in dimensions for type 2. The only simple chemical distinction between molybdenum and tungsten to emerge so far is that in Fe_3MS_4 complexes tungsten is a 'harder' metal centre than molybdenum, forming the $(\mu_2\text{-OMe})_3$ complex $[Fe_6W_2S_8(SPh)_6(OMe)_3]^{3-}$ under the corresponding conditions that produce the $(\mu_2\text{-SPh})_3$ complexes $[Fe_6Mo_2S_8(SPh)_9]^{3-}$ (Christou & Garner 1980*a*).

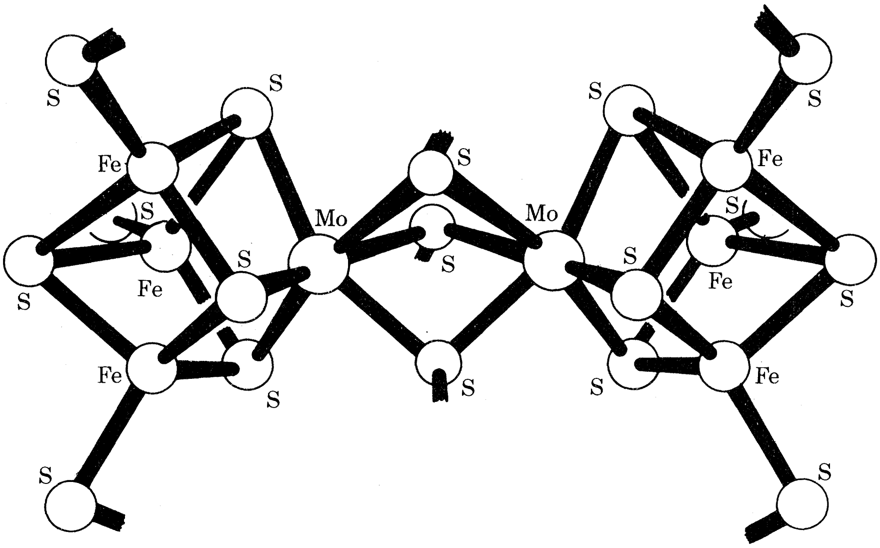

FIGURE 1. Dimeric Fe_3MoS_4 cubane-like framework in $[Fe_6Mo_2S_8(SR)_9]^{3-}$ complexes.

TABLE 1. COMPARISON OF MOLYBDENUM ENVIRONMENTS IN *N*-METHYLFORMAMIDE-EXTRACTED, $S_2O_4^{2-}$ REDUCED, FEMOCO (BURGESS *et al.* 1981; S. D. CONRADSON & K. O. HODGSON, PERSONAL COMMUNICATION 1982) AND $[Fe_6Mo_2S_8(SPh)_9(OMe)_3]^{3-}$

	FeMoco		$[Fe_6Mo_2S_8(SPh)_6(OMe)_3]^{3-}$	
contact	number	distance/Å	number	distance/Å
Mo–O†	3	2.09	3	2.119 (8)
Mo–S	3	2.36	3	2.343 (3)
Mo–Fe	2.8	2.68	3	2.725 (3)

† Mo–O or Mo–N.

The dimensions of the Fe_3MoS_4 cubane-like cluster are essentially independent of the nature of the appended ligands. The number and length of the Mo–S and Mo–Fe contacts within the cubane-like core and, for $[Fe_6Mo_2S_8(SPh)_6(OMe)_3]^{3-}$ (and $[Fe_4MoS_4(SEt)_3(catecholate)_3]^{3-}$), the Mo–O contacts external to the core are in good agreement with the e.X.a.f.s. results of Burgess *et al.* (1981) and S. D. Conradson & K. O. Hodgson, personal communication 1982) (see table 1). The Mo–S and Mo–Fe contacts for the type 1 complexes do not agree with the e.X.a.f.s.

predictions as well as those for type 2; $[Cl_2FeS_2MoS_2FeCl_2]^{2-}$ probably has the closest correspondence observed for a type 1 complex, with 2 Mo–S contacts of 2.204 (5) Å and 2 Mo–Fe of 2.775 (6) Å (Coucouvanis 1981, and references therein).

Electronic structure of Fe_3MoS_4 cubane-like clusters

The total of the formal oxidation states of the metal atoms in the cubane-like cores of the complexes isolated from the $FeCl_3/RS^-/[MoS_4]^{2-}$ reactions is eleven. Thus, the probable description of these oxidation states ranges from $(3Fe^{II}+Mo^{V})$ to $(3Fe^{III}+Mo^{II})$. The application of ^{57}Fe Mössbauer spectroscopy (Christou *et al.* 1980; Holm 1981) has demonstrated that all of the iron atoms are essentially equivalent and have an oxidation state of *ca.* 2.5. This narrows the choice of formal oxidation state descriptions to $(2Fe^{II}Fe^{III}Mo^{IV})$ or $(Fe^{II}2Fe^{III}Mo^{III})$. ^1H n.m.r. spectroscopy is a useful characterization of these clusters and provides a clear indication that $[Fe_6Mo_2S_8(SR)_9]^{3-}$ and related clusters are paramagnets, with most of the paramagnetism being centred on the iron atoms (Christou & Garner 1980*a*).

We have investigated the bulk magnetic susceptibilities and e.p.r. spectra of several of the $[Fe_6M_2S_8L_9]^{3-}$ (M = Mo or W) complexes and interpreted these data in terms of antiferromagnetic spin-coupling between the iron atoms of these complexes; the electrons formally associated with molybdenum (or tungsten) are assumed to be spin-paired, because of electronic exchange interactions across the bridging region or the trigonally distorted octahedral geometry at the metal, or both (Christou *et al.* 1981, 1982). The exchange interaction between the (high-spin) iron atoms,

$$H_{\mathrm{ex}} = -2\alpha J\hat{S}_1\cdot\hat{S}_2 - 2J\hat{S}^*\cdot\hat{S}_3, \qquad (1)$$

within a Fe_3MS_4 cluster is presumed to be the dominant perturbation; centres Fe_1 and Fe_2 are taken to have the same oxidation state and are initially coupled to give a set of resultant spins S^*, which are then coupled with the spin, S_3, of the other iron atom to produce the resultant spin, S', for the individual cube. The inter-cube coupling within an anion (βJ), the applied magnetic field (H) and zero-field splitting (D) have been considered as simultaneous perturbations via the Hamiltonian

$$Hp = \sum_{i=a}^{b}[g_{\parallel}\beta H_z\hat{S}'_z(i) + g_{\perp}\beta\{H_x\hat{S}'_x(i) + H_y\hat{S}'_y(i)\} + D\{\hat{S}'^2_z(i) - \tfrac{1}{3}\hat{S}'(i)(\hat{S}'(i)+1)\}] - 2\beta J\hat{S}'_a\cdot\hat{S}'_b. \qquad (2)$$

Equation (2) was applied to product spin functions $|S'(a)\,M_{\mathrm{S}}(a),\ S'(b)\,M_{\mathrm{S}}(b)\rangle$, zero-order energies of which were taken as the sum of the energies of $S'(a)$ and $S'(b)$ resulting from the application of (1). Also, all possible combinations of $S'(a)$ and $S'(b)$ that would lead to thermally occupied states were considered. Magnetic susceptibilities were calculated (Christou *et al.* 1982) and e.p.r. spectra were simulated (Collison & Mabbs 1982) as described elsewhere. The magnetic properties of $[NEt_4]_3[Fe_6M_2S_8(SPh)_6(OMe)_3]$ (M = Mo or W), $[NBu^n_4]_3[Fe_6Mo_2S_8(SPh)_9]$ and $[NEt_4]_3[Fe_6W_2S_8(SEt)_9]$ has been interpreted by this approach for both the $\{2Fe^{II}Fe^{III}Mo^{IV}\}$ and the $\{Fe^{II}2F^{III}Mo^{III}\}$ models, although rather different values of the parameters, mainly g and α, are required. However, the successful simulation of the solid-state e.p.r. spectra has been achieved only for the $\{2Fe^{II}Fe^{III}Mo^{IV}\}$ model, and then only if a small amount of inter-cube coupling was included. On each cube, only two levels were found to be thermally populated at $\leqslant$ 10 K, one with $S' = \frac{1}{2}$ and another $S' = \frac{3}{2}$. An interpretation, by using the $\{2Fe^{II}Fe^{III}Mo^{IV}\}$

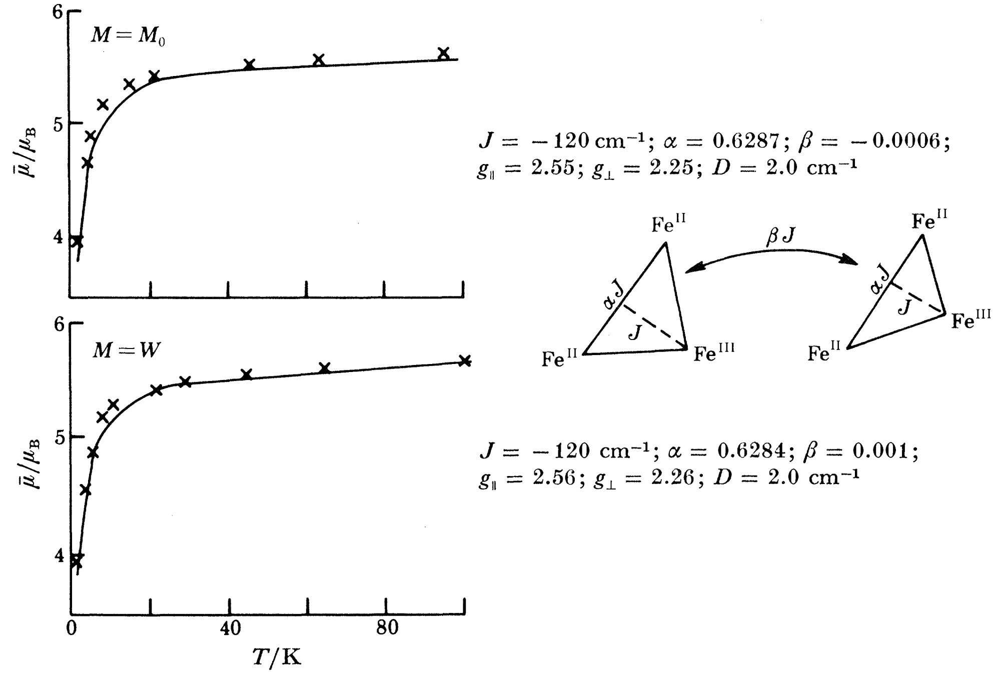

FIGURE 2. Variation of $\bar{\mu}$ with temperature for $[NEt_4]_3[Fe_6M_2S_8(SPh)_6(OMe)_3]$ (M = Mo or W) and interpretation using an antiferromagnetic spin-coupling model within two high-spin $\{2Fe^{II}Fe^{III}\}$ moieties and allowing weak coupling between these groups of iron atoms. ×, Experiment; —, calculation.

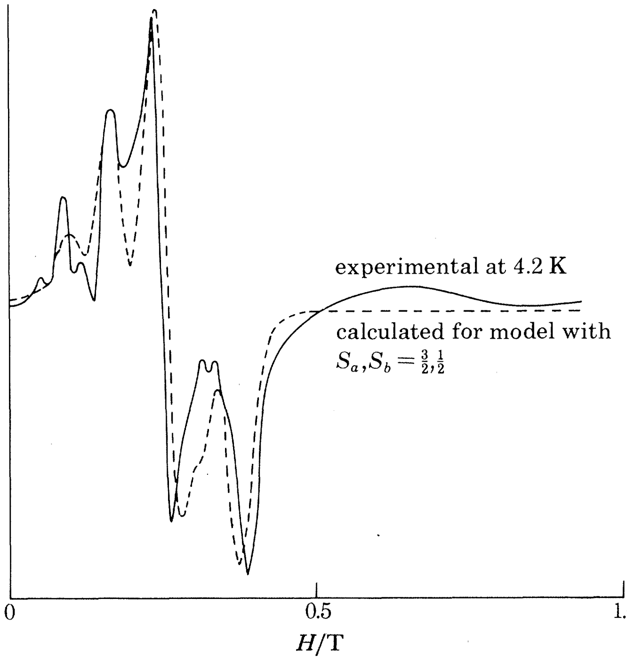

FIGURE 3. E.p.r. spectrum of powdered $[NEt_4]_3[Fe_6W_2S_8(SPh)_6(OMe)_3]$ at 4.2 K (——) and a simulation (– – –), $\nu = 9.238$ GHz ($S_i \beta J S_j$, where $\beta J = -0.03\ \text{cm}^{-1}$, $E_a(S' = \frac{1}{2}) = E_b(S' = \frac{1}{2}) = 0.0\ \text{cm}^{-1}$, $E_a(S' = \frac{3}{2}) = E_b(S' = \frac{3}{2}) = 4.0\ \text{cm}^{-1}$. Line widths for $S'_a = \frac{3}{2}$ coupled with $S'_b = \frac{1}{2}$ (and vice versa), $w_\parallel = 300$ G (30 mT), $w_\perp = 450$ G (45 mT); $S'_a = \frac{1}{2}$ with $S'_b = \frac{1}{2}$, $w_\parallel = w_\perp = 1000$ G (100 mT); $S'_a = \frac{3}{2}$ with $S'_b = \frac{3}{2}$, $w_\parallel = w_\perp = 2000$ G (200 mT); $g_x = g_y = g_z = 2.25$; $D = 2.0\ \text{cm}^{-1}$.

model, of the variation of $\bar{\mu}$ with temperature for the $[NEt_4]_3[Fe_6M_2S_8(SPh)_6(OMe)_3]$ salts is shown in figure 2. For powdered $[NEt_4]_3[Fe_6W_2S_8(SPh)_6(OMe)_3]$ the observed e.p.r. spectrum and a simulated spectrum are shown in figure 3 and the principal features are well reproduced by this model, considering only the coupling of $S_a' = \frac{1}{2}$ with $S_b' = \frac{3}{2}$, and vice versa.

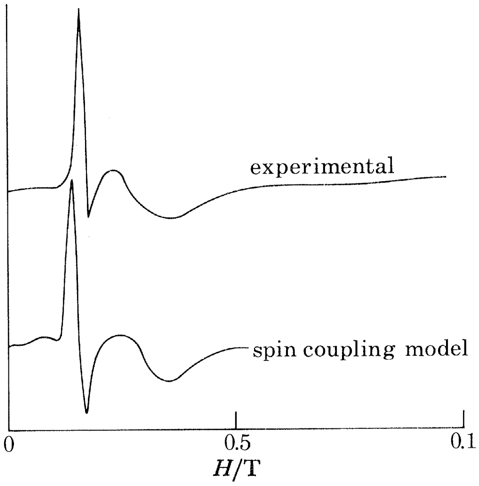

FIGURE 4. E.p.r. spectrum of $[NEt_4]_3[Fe_6W_2S_8(SPh)_6(OMe)_3]$ in MeCN at 10 K, $\nu = 9.238$ GHz; spectrum calculated for $g_\parallel = 2.10$, $g_\perp = 2.20$; $|\beta J| \leqslant 0.012\ \mathrm{cm^{-1}}$; line widths for S_a' = coupled with $S_b' = \frac{3}{2}$, $w_\parallel = 400$ G (40 mT), $w_\perp = 150$ G (15 mT); $S_a' = \frac{3}{2}$ with $S_b' = \frac{1}{2}$, $w_\parallel = w_\perp = 1400$ G (140 mT). Otherwise, parameters the same as for figure 3.

This simple antiferromagnetic coupling model has therefore allowed a reasonably successful interpretation of the magnetic and e.p.r. properties of these Fe_3MS_4 (M = Mo or W) cubane-like dimers. Although these complexes do not display e.p.r. spectra identical to $M_{\mathrm{e.p.r.}}$, we are encouraged by the results obtained, particularly because the appropriate spin states ($S = \frac{3}{2}, \frac{1}{2}$) appear to be involved. The appearance of the e.p.r. spectrum of these complexes can change dramatically from solid state to solution (cf. figures 3 and 4), and this behaviour is attributed to small structural differences perturbing the magnetic couplings or to intermolecular interactions changing the relaxation mechanisms thereby affecting the line widths for the e.p.r. transitions, or both. These observations may be relevant to the significant change in the appearance of the e.p.r. spectrum of the 'molybdenum–iron cluster' of nitrogenase, from methyl ethyl ketone to *N*-methylformamide solution (Shah & Brill 1981). Finally, we note that the e.p.r. spectrum of these molybdenum-containing complexes involve no observable $^{95,97}Mo$ hyperfine couplings and that such couplings are not seen for FeMoco and $M_{\mathrm{e.p.r.}}$; for the chemical systems, this observation is consistent with the assumption that the unpaired electron spin density is localized primarily on the iron atoms.

REDOX PROPERTIES

The redox properties of the Fe_3MS_4 (M = Mo or W) cubane-like complexes have been described in detail elsewhere (Christou *et al.* 1980; Acott *et al.* 1982; Holm 1981); the type 1 complexes have a disappointingly restricted redox chemistry. We merely wish to observe here that each Fe_3MS_4 core, in the oxidation level encountered in $[Fe_6M_2S_8L_9]^{3-}$ complexes, is capable of undergoing one reversible and then one irreversible one-electron reduction, and a reversible one-electron oxidation. The dimeric nature of these complexes results in these electron transfers appearing in pairs (see figure 5), producing potentially valuable two-electron transfers. However, as FeMoco appears to be monomeric in molybdenum, the relevance of the chemical studies to the biochemical system lies in the availability of one state more oxidized and one state more reduced than the e.p.r.-active ($S = \frac{3}{2}$) state.

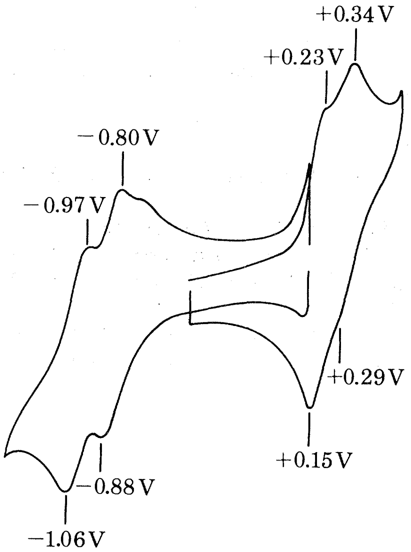

Figure 5. Cyclic voltammogram for $[NEt_4]_3[Fe_6Mo_2S_8(SPh)_6(OMe)_3]$ in MeCN (against standard calomel electrode).

Conclusions

The stimulus of the isolation (Shah & Brill 1977) and e.X.a.f.s. characterization (Cramer *et al.* 1978) of FeMoco has led to the development of an interesting new area of chemistry, that is complexes containing Fe–Mo–S clusters. Of the complex currently characterized, those containing Fe_3MoS_4 cubane-like clusters appear to be the most relevant to FeMoco, in the particular respects of their molybdenum environment, e.p.r. spectra and redox properties. As yet, however, the detailed nature of FeMoco remains obscure, especially in terms of the appended ligands and the location of the iron atoms additional to those within *ca.* 2.7 Å of the molybdenum.

We look forward to further developments in this challenging topic and thank the S.E.R.C. for financial support.

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